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Study of plasma essential element concentrations to explore markers of acute myocardial infarction

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Abstract

Essential element concentrations in biological samples may be related to the pathogenesis of various diseases. Previous studies have reported that serum iron (Fe), zinc (Zn), and copper (Cu) were related to acute myocardial infarction (AMI). However, the differences in element concentrations between AMI and other cardiac disease has not been investigated. In this study, differences in plasma Fe, magnesium (Mg), Zn, Cu, calcium (Ca), inorganic phosphorus (P), and cardiac troponin T (TnT) levels in heart disease patients (AMI, angina, heart failure, and chest pain) were investigated to explore potential markers of AMI. Fe, Mg, Zn, and Cu concentrations were assayed by using a Metallo Assay kit; Ca and inorganic P were determined by using an automatic biochemical analyzer; and cardiac TnT levels were assayed by using enzyme-linked immunosorbent assay. Plasma TnT levels were higher in AMI than in other heart diseases and were negatively correlated with Cu and Ca. Fe, Cu, and inorganic P levels were within the normal range, while Mg and Ca levels were lower, and Zn levels were higher than the normal range in heart disease patients. Except Mg, no significant differences in element levels were observed among heart diseases: Mg levels were significantly higher in AMI than in heart failure. These results suggest that lower Cu and Ca levels and a higher Mg level compared with other heart diseases may be a marker of AMI.

Keywords Heart disease · Essential elements · Markers of acute myocardial infarction · Cardiac troponin T

Introduction

Iron (Fe), zinc (Zn), copper (Cu), calcium (Ca), and magnesium (Mg) are essential elements in the body. They play important roles in metabolic reactions and are essential for physiological enzyme processes (Yuan et al. 2010), with more than 500 enzymes incorporating essential elements in the active center or a prosthetic group (Freeland-Graves et al. 2015). Changes in essential element homeostasis may damage tissue through oxidative stress (redox metabolism imbalance) and inflammation (Morrison et al. 1994; Little et al. 2010; Li et al. 2012). Therefore, essential-element concentrations in biological samples may be related to the pathogenesis of various diseases. Indeed, elemental analysis of human serum may be useful for the diagnosis of phenylketonuria (Gok et al. 2016), bronchial asthma (Vural et al. 2000), and amyotrophic lateral sclerosis (Peters et al. 2016). In addition, higher blood Cu levels and lower Zn levels have been reported in pulmonary tuberculosis (Deveci et al. 2003) and systemic lupus erythematosus (Tóth et al. 2017). Furthermore, higher blood Cu levels have been confirmed in cancer patients (Guo et al. 2012; Stepien et al. 2017; Tamai et al. 2020; Lin et al. 2021; Pala et al. 2022).

In addition to the above diseases, associations have been reported between blood essential element concentrations and heart diseases. An increased risk of fatal myocardial infarction was identified in individuals with high serum Fe concentrations in a Canadian

population (Morrison et al. 1994), while increased Fe, increased Cu, and decreased Zn concentrations were found in the whole blood of Pakistani myocardial infarction patients (Kazi et al. 2008). Low serum and dietary Mg were reported in American patients with cardiovascular disease (Ma et al. 1995) and low serum Zn concentrations were reported in ischemic cardiomyopathy patients in Spain (Mártin-Lagos et al. 1997). Ford (2000) found elevated serum Cu concentrations in American cardiovascular disease patients. In contrast, decreased serum Cu concentration was reported in Russian patients with transient ischemic attack (Klimenko et al. 2016). Decreased serum Fe and/or Zn has been reported in Pakistani patients with acute myocardial infarction (AMI) (Khan et al. 1984; Kazi et al. 2008) In contrast, increased serum Fe levels and risk of fatal AMI (≥175 μg/dL) were reported in a Canadian population (Morrison et al. 1994). Few studies have investigated blood element concentration as a diagnosis marker of AMI. Huang et al. (2014) suggested that low serum Fe concentration is a potential prognostic factor for left ventricular systolic function after revascularization therapy for AMI and may be a novel biomarker for therapeutic intervention. Lim et al. (2023) suggested that Cu and selenium may be specific biomarkers of AMI. Because AMI is the most serious syndrome of coronary heart disease (Mladenka et al. 2009) and one of the leading causes of mortality

and morbidity worldwide (Lim et al. 2023), effective diagnosis markers of AMI are urgently needed.

However, to our knowledge, the differences in blood essential element concentrations among cardiovascular diseases have not been investigated. Therefore, in this study, plasma Fe, Mg, Zn, and Cu concentrations were assayed in patients with heart disease (AMI, angina, heart failure, and chest pain) to elucidate the differences among heart diseases and explore diagnosis markers of AMI. Moreover, plasma calcium (Ca), inorganic phosphorus (P), and cardiac troponin T (TnT) levels were investigated.

Materials and methods

Samples

Venous blood samples were Materials and methods

taken from 52 patients with heart disease who visited the emergency outpatient department of Shimane University Hospital (Shimane, Japan) from 2016 to 2019 (Table 1), including 24 patients with AMI, 8 with angina, 10 with heart failure, and 10 with chest pain. Blood samples were taken from patients upon admission to the hospital. Informed consent was obtained from all patients. Whole blood (7 mL) was drawn into heparinized tubes and plasma was separated by centrifugation at 500 × *g* and stored at −80°C until analysis. This study protocol (20151214-2) was reviewed and approved by

the Human Ethics Committee of Shimane University School of Medicine and the data collection methods were carried out in accordance with the approved guidelines.

Table 1
Sample size and age of each heart disease patient.

	Male (n = 32)	Female (n = 20)	Total (n = 52)
Acute myocardial infarction	14	10	24
Cardiac angina	6	2	8
Cardiac failure	7	3	10
Chest pain	5	5	10
Age, years	72.8 (30–88)	76.1 (45–89)	74.0 (64–80)

TnT assay

Cardiac TnT levels in plasma (50 µL) were determined by enzyme-linked immunosorbent assay (ELISA) using a Human Cardiac Troponin T ELISA Kit (Abcam, San Diego, CA). Absorbance at 450 nm was measured using a Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA).

Fe, Mg, Zn, and Cu assays

Plasma Fe concentration in the samples (40 μL) was assayed using a Metallo Assay Iron Assay kit LS - Ferrozine Method (AKJ Global Technology Co., Ltd., Chiba, Japan). Dissociated Fe (Fe^{2+} and Fe^{3+}) and Ferrozine (chelator) form a purple complex and the absorbance at 560 nm was measured by using a Multiskan™ GO Microplate Spectrophotometer to determine the Fe concentration. A Metallo Assay Magnesium Assay kit LS - Xylidyl Blue-I Method (AKJ Global Technology Co., Ltd.) was used to analyze the plasma Mg concentration in the samples (3 μL). The Mg-Xylidyl Blue-I complex (chelator) form a red-colored complex, and the absorbance at 660 nm was measured to determine the Mg concentration. Dissociated Zn was analyzed by using a Zinc Assay kit LS - 5-Br-PAPS Method (AKJ Global Technology Co., Ltd.). Zn ions form a red-colored complex with 5-Br-PAPS (as chelator). Zn concentrations in the samples (12 μL) were determined by measurement of the absorbance at 560 nm, with correction for absorbance at 700 nm. A Copper Assay kit LS - 3,5-DiBr-PAESA Method (AKJ Global Technology Co., Ltd.) was used to measure plasma Cu levels in the samples (12 μL). Dissociated Cu^{+} forms a brown 3-5 DiBr-PAESA complex (as chelator) and the absorbance at 580 nm was measured to determine Cu levels.

Ca and P assays

Plasma Ca and inorganic P levels were determined using an automated biochemical analyzer (SPOTCHEM SP4430; Arkray Inc., Kyoto, Japan) with reagent strips (Spotchem II Ca2 and IP; Arkray Inc.) according to the manufacturer's instructions. In this assay, 250 μL of plasma sample was used.

Statistical analysis

Between-group differences in essential-element concentrations and TnT levels were analyzed using the Tukey–Kramer test. Spearman's rank correlation test was performed to examine the relationships between the plasma levels of TnT and essential elements. These analyses were conducted using Bell Curve for Excel (Social Survey Research Information Co. Ltd., Tokyo, Japan).

Results and Discussion

Plasma TnT levels in heart disease patients

Levels of plasma TnT, which is a marker of myocardial infarction, were investigated by using Human Cardiac Troponin T ELISA Kit in heart disease patients (Fig. 1). In AMI, plasma TnT levels in 4 patients out of 14 patients greatly exceeded the normal limit. In addition, plasma TnT levels were higher than the normal limit (<14 pg/mL) in about half of the other heart disease patients. Although significant differences were not observed, TnT levels were higher in AMI patients than in patients with other heart diseases.

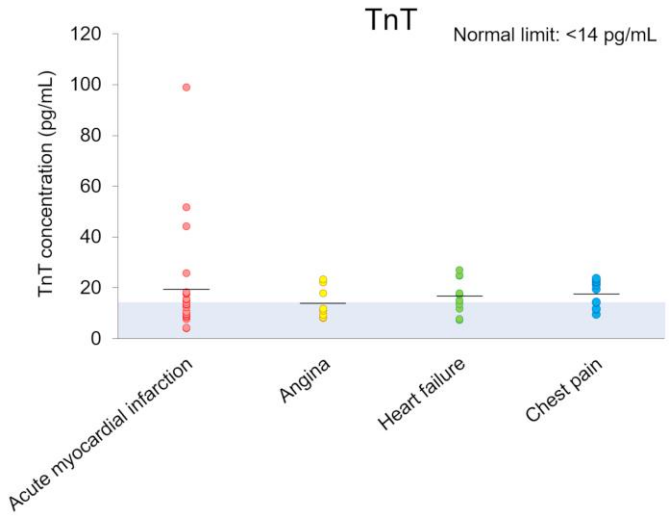

Fig. 1. Dot plot of plasma TnT levels (pg/mL) in heart disease patients. Average is shown as a bar. A Tukey–Kramer test did not reveal significant between-group differences. The light blue area shows the normal range.

Plasma Fe concentrations in heart disease patients, which were assayed by using a Metallo Assay Iron Assay kit LS, are shown in Figure 2. Fe is utilized mainly for heme synthesis and incorporation into hemoglobin. Body Fe status is associated with cardiovascular disease development: iron-deficiency anemia has been reported to be most common in heart failure patients (Naito et al. 2021). Previous studies reported an association between increased serum Fe levels and risk of fatal AMI (≥175 µg/dL) (Morrison et al. 1994), myocardial infarction (Kazi et al. 2008), and acute coronary syndrome (Altekin et al. 2005) but also decreased Fe levels in AMI (Khan et al. 1984). A recent study demonstrated that high serum Fe levels within the normal range are associated with lower cardiovascular disease incidence (Gutierrez-Bedmar et al. 2021). In this study, almost all the Fe concentrations in both men and women were within the

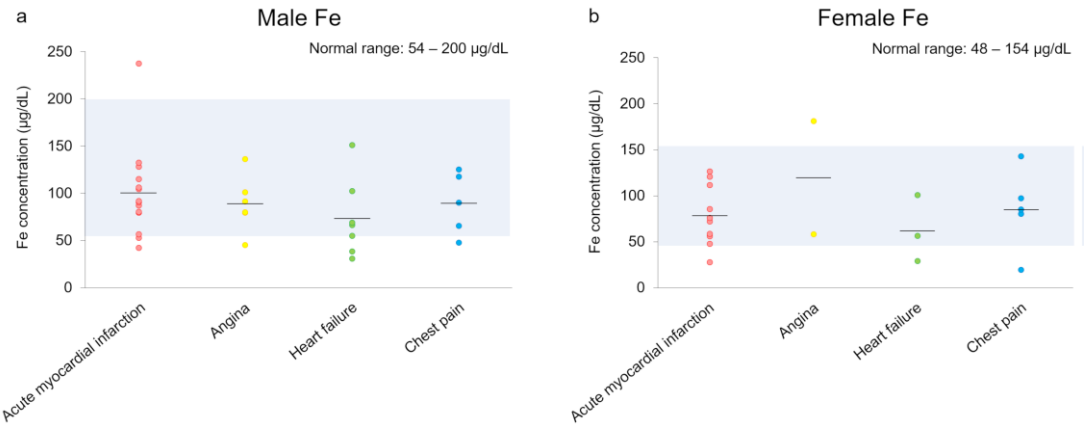

Fig. 2. Dot plot of plasma (a) male Fe and (b) female Fe concentrations (µg/dL) in heart disease patients. Average is shown as a bar. A Tukey–Kramer test did not reveal significant between-group differences. The light blue area shows the normal range.

normal range (male: 54–200 µg/dL; female: 48–154 µg/dL) and no significant differences were observed among the heart diseases (Fig. 2a, b).

Mg plays a role in maintaining electrochemical gradients, and cardiac excitation is affected by Mg modulation of Na and Ca channels and the Na/K ATPase pump of cardiac myocytes (Tangvoraphonkchai et al. 2018). Mg has anti-inflammatory effects (Swaminathan et al. 2007; Shi et al. 2008). A low serum Mg concentration has been implicated in cardiovascular diseases (Ma et al. 1995; Gorący et al. 2021), the pathophysiology of heart failure (Tangvoraphonkchai et al. 2018), and valve calcification caused by an imbalance between calcification inhibitors and promoters, elevated oxidative stress, and the inflammatory response (Tsai et al. 2023). Plasma Mg

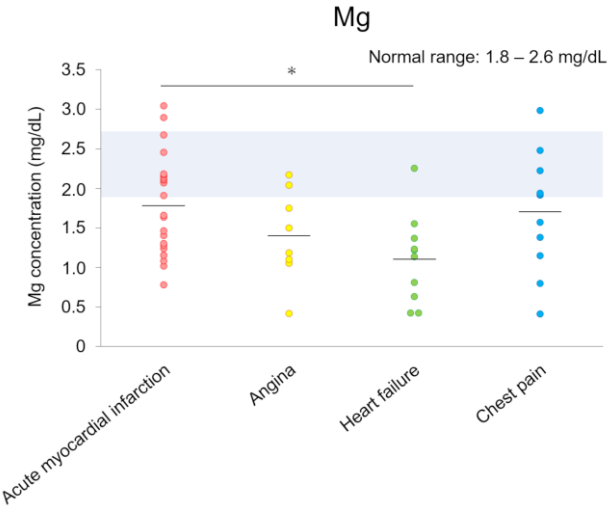

Fig. 3. Dot plot of plasma Mg concentration (mg/dL) in heart disease patients. Average is shown as a bar. Data were analyzed with a Tukey–Kramer test. *$p < 0.05$ when compared between individual groups. The light blue area shows the normal range.

concentrations in heart disease patients, which were assayed by using a Metallo Assay Magnesium Assay kit LS, are shown in Figure 3. Similar to previous studies (Ma et al. 1995; Gorący et al. 2021), heart disease patients showed lower Mg concentrations than normal range (1.8–2.6 mg/dL) and a significant difference was observed between AMI and heart failure patients ($p < 0.05$).

Zn, which is an important component of bio-membranes and a vital cofactor of various enzymes, plays an antioxidant role (Bray and Bettger 1990). Zn is an essential metallic micronutrient that is potentially associated with cardiovascular diseases (Little et al. 2010). Zn is indispensable for cardiovascular physiology and plays a protective role in coronary artery disease and cardiomyopathy (Little et al. 2010; Ozyildirim et al. 2023). Previous studies reported decreased serum Zn levels in AMI (Khan et al. 1984; Kazi et al. 2008), acute and chronic heart failure (Alexanian et al. 2014), acute coronary syndrome (Altekin et al. 2005; Bayır et al. 2013), heart failure (Yoshihisa et al. 2018; Yu et al. 2018), and cardiovascular disease (Ma et al. 1995). Plasma Zn concentrations in heart disease patients, which were assayed by using a Zinc Assay kit LS, are shown in Figure 4. In the present study, Zn concentrations in heart disease patients were above the normal range (80–130 μg/dL) and there were no significant differences among the different heart diseases, in contrast to previous studies.

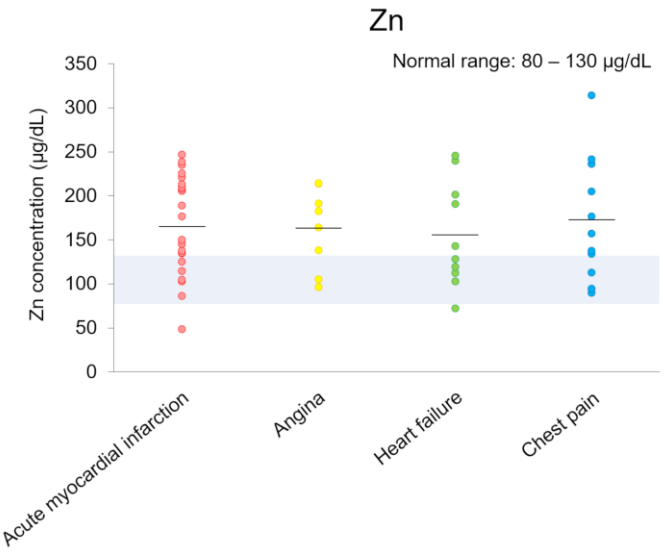

Fig. 4. Dot plot of plasma Zn concentration (µg/dL) in heart disease patients. Average is shown as a bar. A Tukey–Kramer test did not reveal significant between-group differences. The light blue area shows the normal range.

Cu is an essential element for super oxide dismutase (SOD), which catalyzes the oxidation-reduction reaction (Linder et al. 1996) and also requires Zn and manganese to function (Kazi et al. 2008). SOD is a powerful antioxidant defense enzyme that eliminates highly reactive superoxide. However, excess circulating Cu may promote reactive oxygen species (Bo et al. 2008). In addition, a higher Cu concentration may increase cardiovascular risk due to its potential role in atherogenesis (Salonen et al. 1991; Kang 2011). Elevated serum Cu levels have been reported in cardiovascular disease (Khan et al. 1984; Kazi et al. 2008; Ford 2000; Isiozor et al. 2023), acute and chronic heart failure (Alexanian et al. 2014), and acute coronary syndrome (Altekin et al. 2005). In contrast, decreased serum Cu concentrations have been found in chronic heart

failure patients and acute coronary syndrome patients (Bayır et al. 2013). The plasma Cu concentrations in heart disease patients, which were assayed using a Copper Assay kit LS, are shown in Figure 5. In this study, the average plasma Cu levels in the heart disease patients were within the normal range (76–140 µg/dL), although several patients, particularly those with chest pain, showed higher levels. Nonetheless, significant differences were not observed in Cu levels among the different heart diseases.

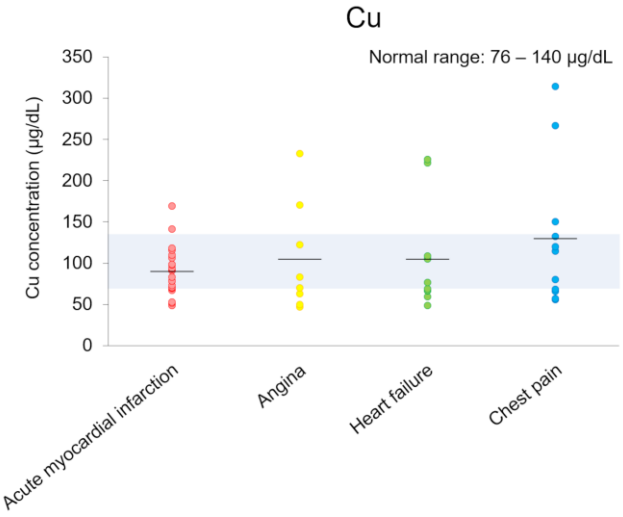

Fig. 5. Dot plot of plasma Cu concentration (µg/dL) in heart disease patients. Average is shown as a bar. A Tukey–Kramer test did not reveal significant between-group differences. The light blue area shows the normal range.

Plasma Cu/Zn ratio in heart disease patients

As mentioned above, Cu and Zn are essential elements that are important cofactors for SOD, which prevents free-radical-induced injury (Yücel et al. 1994). Cu and Zn in the body interact with and balance each other (Bayır et al. 2013). Relatively high Cu and low Zn circulating levels may result in increased oxidative stress or impaired antioxidant

capacity (Bo et al. 2008). Elevated Cu/Zn ratio has been reported in cancer patients (Stepien et al. 2017; Bengtsson et al. 2023) and pulmonary disease patients (Kunutsor et al. 2023). Both excess Cu and Zn deficiency have been linked to type 2 diabetes with renal dysfunction (Hamasaki et al. 2016) and coronary heart events in type 2 diabetes (Soinio et al. 2007; Yary et al. 2016). Moreover, imbalance between Cu and Zn may be a factor in the etiology of cardiovascular disease (Malavolta et al. 2010) and elevated Cu/Zn ratio has been reported in cardiovascular disease patients (Little et al. 2010; Ford 2000; Malavolta et al. 2010; Mirończuk et al. 2021; Kunutsor et al. 2022). The serum Cu/Zn ratio in healthy individuals is about 1 (Suciu et al. 1992). However, in this study, the Cu/Zn ratio of heart disease patients was lower than 1. Significant differences were not observed among heart disease patients (Fig. 6).

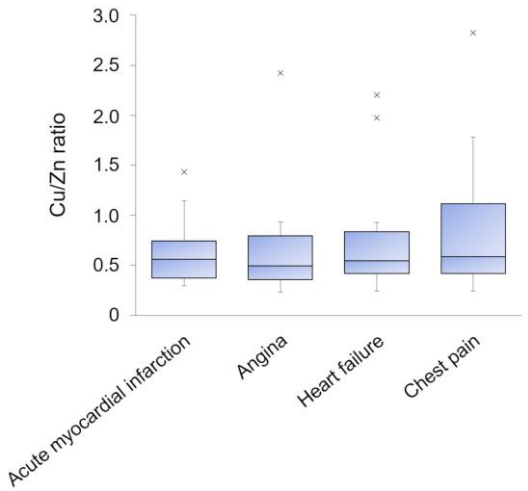

Fig. 6. Box plot of plasma Cu/Zn ratio of heart disease patients. A Tukey–Kramer test did not reveal significant between-group differences.

Plasma Ca and inorganic P levels in heart disease patients

Lower serum Ca levels are associated with an increased risk of sudden cardiac arrest (Yarmohammadi et al. 2017). High serum P levels have been connected to adverse health outcomes in cardiovascular diseases due to artery calcification (Adeney et al. 2009; Giachelli 2009; Rajamannan 2011; Raikou 2021). In addition to the Fe, Mg, Zn, and Cu, we measured plasma Ca and P levels in heart disease patients, using automatic biochemical analyzer (Fig. 7). Plasma Ca levels were lower than the normal range (8.8–10.4 mg/dL) in patients with heart diseases and no differences were observed among the heart diseases (Fig. 7a). Plasma inorganic P levels were within the normal range but relatively low in heart disease patients, and patients with chest pain showed lower levels than the normal range (2.5–4.5 mg/dL) while two patients with AMI showed high levels

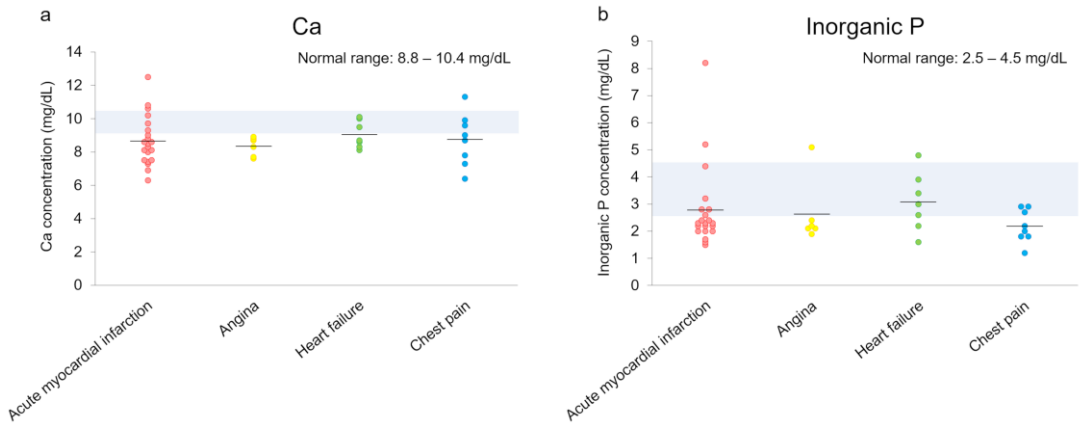

Fig. 7. Dot plot of plasma (a) Cu and (b) P concentrations (mg/dL) in heart disease patients. Average is shown as a bar. A Tukey–Kramer test did not reveal significant between-group differences. The light blue area shows the normal range.

(Fig. 7b).

Correlation analysis

Table 2 shows the results of the Spearman's rank correlation test that examined the relationship between the plasma levels of the TnT and essential elements. Correlation analyses between cardiac markers and essential element levels are scarce. A previous study reported a positive correlation between serum Cu and cardiac markers (cardiac TnT, cardiac troponin I, and creatine kinase-MB), a positive correlation between serum Fe and troponin I, and a negative correlation between serum Zn and cardiac TnT (Altekin et al. 2005). In this study, serum TnT levels were negatively correlated with Cu ($p < 0.05$) and Ca ($p < 0.01$) in AMI patients (Table 2).

Jariwala et al. (2014) indicated a positive correlation of Fe and Zn concentrations in maternal serum and cord blood serum. In patients with chronic heart failure, moderate and positive correlations were observed for Fe vs Mg and Fe vs Cu in men and for Fe vs Ca in women, while weak positive correlations were observed for Fe vs Cu, Fe vs Zn, and Fe vs inorganic P in women (Swaminathan et al. 2007). Similarly, in this study, Fe levels were positively correlated with Cu in AMI patients ($p < 0.05$) and heart failure patients ($p < 0.01$) and were correlated with Ca ($p < 0.05$) in AMI patients (Table 2).

Al-Jameil et al. (2017) reported a negative correlation for Mg vs Ca and Mg vs Zn in the serum of preeclampsia patients. In patients with chronic heart failure, strong positive correlations have been observed for Mg vs Cu and Mg vs Ca in men and women, while a negative correlation has been observed for Mg vs inorganic P in men (Gorący et al. 2021). In the present study, plasma Mg levels were positively correlated with Ca ($p < 0.05$) and inorganic P ($p < 0.05$) in patients with heart failure (Table 2).

Lee et al. (2019) indicated a positive correlation between the serum concentrations of Zn and Cu in patients in an intensive care unit. In patients with chronic heart failure, a moderate positive correlation has been observed for serum Zn vs Ca concentration in women (Gorący et al. 2021). In contrast to the previous study, we observed a negative correlation for Zn vs Ca ($p < 0.05$) in the serum of patients with chest pain (Table 2). A negative correlation was observed for Zn vs inorganic P in patients with AMI. Ca levels were positively correlated with P in AMI, angina, and heart failure patients ($p < 0.05$).

Table 2 Spearman's rank correlation analysis of the Fe, Mg, Zn, Cu, Ca, P, and TnT levels in heart disease patients.

	Acute myocardial infarction	Angina	Heart failure	Chest pain
TnT–Fe	−0.4111	−0.3810	−0.4909	−0.2455
TnT–Mg	−0.0049	−0.5238	−0.3526	0.1000
TnT–Zn	−0.0247	−0.0714	0.0667	−0.2909
TnT–Cu	**−0.4407** *	−0.6667	−0.4061	−0.2636
TnT–Ca	**−0.5719** **	0.6000	0.0714	−0.2619
TnT–P	−0.2237	0.3189	−0.1786	−0.5663
Fe–Mg	−0.5719	−0.4524	0.4255	−0.5091
Fe–Zn	−0.1689	0.3810	−0.0909	−0.0182
Fe–Cu	**0.4407** *	0.3571	**0.8909** **	−0.0727
Fe–Ca	**0.4536** *	−0.5429	0.3929	−0.1429
Fe–P	−0.0228	−0.0290	0.7143	0.2771
Mg–Zn	−0.0721	−0.2381	0.0061	0.4909
Mg–Cu	0.1037	0.0476	0.3526	0.0000
Mg–Ca	−0.1748	−0.4857	**0.8214** *	−0.5476
Mg–P	−0.1233	−0.5508	**0.7857** *	−0.1325
Zn–Cu	0.1502	−0.2619	−0.2485	0.0364
Zn–Ca	−0.2495	−0.0857	−0.2500	**−0.7857** *
Zn–P	**−0.5316** *	0.0580	−0.2143	0.0241
Cu–Ca	0.2912	−0.1429	0.2143	0.2857
Cu–P	0.0313	0.0290	0.5714	0.6627
Ca–P	**0.4747** *	**0.8117** *	**0.8214** *	0.4579

Cardiac troponin T concentration was assayed using a Human Cardiac Troponin T ELISA Kit; plasma Fe concentration was assayed using a Metallo Assay Iron Assay kit LS; plasma Mg concentration was assayed using a Metallo Assay Magnesium Assay kit LS; plasma Zn concentration was assayed using a Zinc Assay kit LS; plasma Cu concentration was assayed using a Copper Assay kit LS; Plasma Ca and inorganic P levels in 250 μL plasma were determined using a SPOTCHEM automatic biochemical analyzer.

Values in **bold** indicate statistical significance.

*$p < 0.05$; **$p < 0.01$ by Spearman's rank correlation test.

Conclusions

In this study, we examined plasma TnT, Fe, Mg, Zn, Cu, Ca, and inorganic P

levels in patients with heart disease (AMI, angina, heart failure, and chest pain). Plasma Fe, Cu, and inorganic P levels were within the normal range, while Mg and Ca levels were lower, and Zn levels were higher than the normal range in all heart disease patients. Higher plasma TnT levels were observed in AMI patients and were negatively correlated with Cu and Ca. Mg levels were significantly higher in AMI than in heart failure. In other element levels, no significant differences were observed among heart diseases. These preliminary results suggest that lower Cu and Ca levels and a higher Mg level than those of other heart diseases may be a marker of AMI.

The novelty of the present study is that it elucidates the differences in plasma element concentrations among cardiovascular disease patients to discriminate AMI from other cardiac diseases. The limitations of the study include the small number of patients having each cardiac disease, the fact that only four cardiac diseases were investigated, and that fasting conditions were not taken into consideration. Therefore, further confirmation of these data using larger patient samples, investigating other cardiac diseases, and considering fasting conditions are needed to verify whether lower Cu and Ca levels and a higher Mg level in the plasma of AMI patients are indeed a marker of AMI.

Author contributions JF: Writing – original draft, review and editing, visualization. NN: Writing – review and editing, and visualization. YT: Resources

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Data availability No dataset was generated or analyzed during the present study.

Statements and Declarations

Competing Interests There are no conflicts of interest of declare.

Ethical approval The study including usage of plasma derived from patients and control subjects was reviewed and approved by the Human Ethics Committee of Shimane University School of Medicine (approval number: 20151214-2).

Consent to participate Informed consent was obtained from all participants included in the present study.

References

Adeney KL, Siscovick DS, Ix JH et al (2009) Association of serum phosphate with vascular and valvular calcification in moderate CKD. J Am Soc Nephrol 20(2):381-387. https://doi.org/10.1681/ASN.2008040349

Alexanian I, Parissis J, Farmakis D et al (2014) Clinical and echocardiographic correlates of serum copper and zinc in acute and chronic heart failure. Clin Res Cardiol103(11):938-949. https://doi.org/10.1007/s00392-014-0735-x

Al-Jameil N, Tabassum H, Ali MN, Qadeer MA, Khan FA, Al-Rashed M (2017). Correlation between serum trace elements and risk of preeclampsia: A case controlled study in Riyadh, Saudi Arabia. Saudi J Biol Sci 24(6):1142-1148. https://doi.org/10.1016/j.sjbs.2015.02.009

Altekin E, Coker C, Sişman AR, Onvural B, Kuralay F, Kirimli O (2005) The relationship between trace elements and cardiac markers in acute coronary syndromes. J Trace Elem Med Biol 18(3):235-242. https://doi.org/10.1016/j.jtemb.2004.12.002

Bayır A, Kara H, Kıyıcı A, Oztürk B, Akyürek F (2013) Levels of selenium, zinc, copper, and cardiac troponin I in serum of patients with acute coronary syndrome. Biol Trace Elem Res 154(3):352-356. https://doi.org/10.1007/s12011-013-9754-0

Bengtsson Y, Demircan K, Vallon-Christersson J et al (2023) Serum copper, zinc and copper/zinc ratio in relation to survival after breast cancer diagnosis: A prospective multicenter cohort study. Redox Biol63:102728. https://doi.org/10.1016/j.redox.2023.102728

Bo S, Durazzo M, Gambino R et al (2008) Associations of dietary and serum copper with inflammation, oxidative stress, and metabolic variables in adults. J Nutr 138(2):305-310. https://doi.org/10.1093/jn/138.2.305

Bray TM, Bettger WJ (1990) The physiological role of zinc as an antioxidant. Free Radic Biol Med 8(3):281-291. https://doi.org/10.1016/0891-5849(90)90076-u

Deveci F, Ilhan N (2003) Plasma malondialdehyde and serum trace element concentrations in patients with active pulmonary tuberculosis. Biol Trace Elem Res 95(1):29-38. https://doi.org/10.1385/BTER:95:1:29

Ford ES (2000) Serum copper concentration and coronary heart disease among US adults. Am J Epidemiol 151(12):1182-1188. https://doi.org/10.1093/oxfordjournals.aje.a010168

Freeland-Graves JH, Sanjeevi N, Lee JJ (2015) Global perspectives on trace element requirements. J Trace Elem Med Biol 31:135-141. https://doi.org/10.1016/j.jtemb.2014.04.006

Giachelli CM (2009) The emerging role of phosphate in vascular calcification. Kidney Int 75(9):890-897. https://doi.org/ 10.1038/ki.2008.644

Gok F, Ekin S, Dogan M (2016) Evaluation of trace element and mineral status and related to levels of amino acid in children with phenylketonuria. Environ Toxicol Pharmacol 45:302-308. https://doi.org/10.1016/j.etap.2016.06.014

Gorący I, Rębacz-Maron E, Korbecki J, Gorący J (2021) Concentrations of Mg, Ca, Fe, Cu, Zn, P and anthropometric and biochemical parameters in adults with chronic heart failure. Peer J 9:e12207. https://doi.org/10.7717/peerj.12207

Guo KF, Zhang Z, Wang JY et al (2012) Variation of urinary and serum trace elements (Ca, Zn, Cu, Se) in bladder carcinoma in China. Asian Pac J Cancer Prev 13(5):2057-2061. https://doi.org/10.7314/apjcp.2012.13.5.2057

Gutierrez-Bedmar M, Olmedo P, Gil F et al (2021) Low serum iron levels and risk of cardiovascular disease in high risk elderly population: Nested case-control study in the PREvención con DIeta MEDiterránea (PREDIMED) trial. Clin Nutr 40(2):496-504. https://doi.org/10.1016/j.clnu.2020.05.044

Hamasaki H, Kawashima Y, Yanai H (2016) Serum Zn/Cu Ratio Is Associated with renal function, glycemic control, and metabolic parameters in Japanese patients with and without Type 2 Diabetes: A Cross-sectional Study. Front Endocrinol (Lausanne)

7:147. https://doi.org/10.3389/fendo.2016.00147

Isiozor NM, Kunutsor SK, Vogelsang D, Isiozor I, Kauhanen J, Laukkanen JA (2023) Serum copper and the risk of cardiovascular disease death in Finnish men. Nutr Metab Cardiovasc Dis 33(1):151-157. https://doi.org/10.1016/j.numecd.2022.09.024

Jariwala M, Suvarna S, Kiran Kumar G, Amin A, Udas AC (2014) Study of the concentration of trace elements Fe, Zn, Cu, Se and their correlation in maternal serum, cord serum and colostrums. Indian J Clin Biochem 29(2):181-188. https://doi.org/10.1007/s12291-013-0338-8

Kang YJ (2011) Copper and homocysteine in cardiovascular diseases. Pharmacol Ther 129(3):321-331. https://doi.org/10.1016/j.pharmthera.2010.11.004

Kazi TG, Afridi HI, Kazi N et al (2008) Distribution of zinc, copper and iron in biological samples of Pakistani myocardial infarction (1st, 2nd and 3rd heart attack) patients and controls. Clin Chim Acta 389(1-2):114-119. https://doi.org/10.1016/j.cca.2007.12.004

Khan SN, Rahman MA, Samad A (1984) Trace elements in serum from Pakistani patients with acute and chronic ischemic heart disease and hypertension. Clin Chem 30(5):644-648.

Klimenko LL, Skalny AV, Turna AA et al (2016) Serum trace element profiles,

prolactin, and cortisol in transient ischemic attack patients. Biol Trace Elem Res 172(1):93-100. https://doi.org/ 10.1007/s12011-015-0586-y

Kunutsor SK, Voutilainen A, Kurl S, Laukkanen JA (2022) Serum copper-to-zinc ratio is associated with heart failure and improves risk prediction in middle-aged and older Caucasian men: A prospective study. Nutr Metab Cardiovasc Dis 32(8):1924-1935. https://doi.org/10.1016/j.numecd.2022.05.005

Kunutsor SK, Voutilainen A, Laukkanen JA (2023) Serum copper-to-zinc ratio and risk of chronic obstructive pulmonary disease: A cohort study. Lung 201(1):79-84. https://doi.org/10.1007/s00408-022-00591-6

Lee YH, Bang ES, Lee JH et al (2019) Serum concentrations of trace elements zinc, copper, selenium, and manganese in critically Ill patients. Biol Trace Elem Res 188(2):316-325. https://doi.org/10.1007/s12011-018-1429-4

Li YV, Zhang JH (2012) Metal ions in stroke pathophysiology. In: Li Y, Zhang J. (eds) Metal ion in stroke. Springer series in translational stroke research. Springer, New York, NY. https://doi.org/10.1007/978-1-4419-9663-3_1

Lim SY, Dayal H, Seah SJ et al (2023) Plasma metallomics reveals potential biomarkers and insights into the ambivalent associations of elements with acute myocardial infarction. J Trace Elem Med Biol 77:127148.

https://doi.org/10.1016/j.jtemb.2023.127148

Lin S, Yang H (2021) Ovarian cancer risk according to circulating zinc and copper concentrations: A meta-analysis and Mendelian randomization study. Clin Nutr 40(4):2464-2468. https://doi.org/10.1016/j.clnu.2020.10.011

Linder MC, Hazegh-Azam M (1996) Copper biochemistry and molecular biology. Am J Clin Nutr 63(5):797S-811S. https://doi.org/10.1093/ajcn/63.5.797

Little PJ, Bhattacharya R, Moreyra AE, Korichneva IL (2010) Zinc and cardiovascular disease. Nutrition 26(11-12):1050-1057. https://doi.org/10.1016/j.nut.2010.03.007

Ma J, Folsom AR, Melnick SL et al (1995) Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: the ARIC study. Atherosclerosis Risk in Communities Study. J Clin Epidemiol 48(7):927-940. https://doi.org/10.1016/0895-4356(94)00200-a

Mladenka P, Hrdina R, Bobrovová Z et al (2009) Cardiac biomarkers in a model of acute catecholamine cardiotoxicity. Hum Exp Toxicol 28(10):631-640. https://doi.org/10.1177/0960327109350665.

Malavolta M, Giacconi R, Piacenza F et al (2010) Plasma copper/zinc ratio: an inflammatory/nutritional biomarker as predictor of all-cause mortality in elderly population. Biogerontology 11(3):309-319. https://doi.org/10.1007/s10522-009-9251-

1

Mártin-Lagos F, Navarro-Alarcón M, Terrés-Martos C, López-G de la Serrana H, López-Martínez MC (1997) Serum copper and zinc concentrations in serum from patients with cancer and cardiovascular disease. Sci Total Environ 204(1):27-35. https://doi.org/10.1016/s0048-9697(97)00163-0

Mirończuk A, Kapica-Topczewska K, Socha K et al (2021) Selenium, copper, zinc concentrations and Cu/Zn, Cu/Se, molar ratios in the serum of patients with acute ischemic stroke in northeastern poland-a new insight into stroke pathophysiology. Nutrients 13(7):2139. https://doi.org/10.3390/nu13072139

Morrison HI, Semenciw RM, Mao Y, Wigle DT (1994) Serum iron and risk of fatal acute myocardial infarction. Epidemiology 5(2):243-246. https://doi.org/10.1097/00001648-199403000-00015

Naito Y, Masuyama T, Ishihara M (2021) Iron and cardiovascular diseases. J Cardiol 77(2):160-165. https://doi.org/ 10.1016/j.jjcc.2020.07.009

Ozyildirim S, Baltaci SB (2023) Cardiovascular diseases and zinc. Biol Trace Elem Res 201(4):1615-1626. https://doi.org/10.1007/s12011-022-03292-6

Pala V, Agnoli C, Cavalleri A et al (2022) Prediagnostic levels of copper and zinc and breast cancer risk in the ORDET cohort. Cancer Epidemiol Biomarkers Prev

31(6):1209-1215. https://doi.org/ 10.1158/1055-9965

Peters TL, Beard JD, Umbach DM et al (2016) Blood levels of trace metals and amyotrophic lateral sclerosis. Neurotoxicology 54:119-126. https://doi.org/10.1016/j.neuro.2016.03.022

Raikou VD (2021) Serum phosphate and chronic kidney and cardiovascular disease: Phosphorus potential implications in general population. World J Nephrol 10(5):76-87. https://doi.org/10.5527/wjn.v10.i5.76

Rajamannan NM (2011) Serum phosphate concentrations: a novel pre-clinical biomarker for cardiovascular calcification. J Am Coll Cardiol 58(3):298-299. https://doi.org/10.1016/j.jacc.2011.01.047

Salonen JT, Salonen R, Korpela H, Suntioinen S, Tuomilehto J (1991) Serum copper and the risk of acute myocardial infarction: a prospective population study in men in eastern Finland. Am J Epidemiol 134(3):268-276. https://doi.org/10.1093/oxfordjournals.aje.a116080

Shi ZM, Hu XS, Yuan BJ, Gibson R, Dai Y, Garg M (2008) Association between magnesium: iron intake ratio and diabetes in Chinese adults in Jiangsu Province. Diabet Med 25(10):1164-1170. https://doi.org/10.1111/j.1464-5491.2008.02558.x

Soinio M, Marniemi J, Laakso M, Pyörälä K, Lehto S, Rönnemaa T (2007) Serum zinc

level and coronary heart disease events in patients with type 2 diabetes. Diabetes Care 30(3):523-528. https://doi.org/10.2337/dc06-1682

Stepien M, Jenab M, Freisling H et al (2017) Pre-diagnostic copper and zinc biomarkers and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition cohort. Carcinogenesis 38(7):699-707. https://doi.org/10.1093/carcin/bgx051

Suciu A, Chirulescu Z, Zeana C, Pîrvulescu R (1992) Study of serum ceruloplasmin and of the copper/zinc ratio in cardiovascular diseases. Rom J Intern Med 30(3):193-200.

Swaminathan S, Fonseca VA, Alam MG, Shah SV (2007) The role of iron in diabetes and its complications. Diabetes Care 30(7):1926-3193. https://doi.org/10.2337/dc06-2625

Tamai Y, Iwasa M, Eguchi A et al (2020) Serum copper, zinc and metallothionein serve as potential biomarkers for hepatocellular carcinoma. PLoS One 15(8):e0237370. https://doi.org/10.1371/journal.pone.0237370

Tangvoraphonkchai K, Davenport A (2018) Magnesium and cardiovascular disease. Adv Chronic Kidney Dis. 25(3):251-260. https://doi.org/10.1053/j.ackd.2018.02.010.

Tóth CN, Baranyai E, Csípő I et al (2017) Elemental analysis of whole and protein

separated blood serum of patients with systemic lupus erythematosus and Sjögren's syndrome. Biol Trace Elem Res 179(1):14-22. https://doi.org/10.1007/s12011-017-0945-y

Tsai S, Li Y, Wu X (2023) Serum magnesium level and cardiac valve calcification in hemodialysis patients. BMC Cardiovasc Disord 23(1):610. https://doi.org/10.1186/s12872-023-03662-5

Yarmohammadi H, Uy-Evanado A, Reinier K et al (2017) Serum calcium and risk of sudden cardiac arrest in the general population. Mayo Clin Proc 92(10):1479-1485. https://doi.org/10.1016/j.mayocp.2017.05.028

Yary T, Virtanen JK, Ruusunen A, Tuomainen TP, Voutilainen S (2016) Serum zinc and risk of type 2 diabetes incidence in men: The Kuopio ischaemic heart disease risk factor study. J Trace Elem Med Biol 33:120-124. https://doi.org/10.1016/j.jtemb.2015.11.001

Yoshihisa A, Abe S, Kiko T et al (2018) Association of serum zinc level with prognosis in patients with heart failure. J Card Fail 24(6):375-383. https://doi.org/10.1016/j.cardfail.2018.02.011

Yu X, Huang L, Zhao J et al. (2018) The Relationship between serum zinc level and heart failure: a Meta-analysis. Biomed Res Int 25;2018:2739014.

https://doi.org/10.1155/2018/2739014

Yuan WA, Yu XJ, Liu FQ, Wang HP, Wang D, Lai XP (2010) Effects of trace element supplementation on the inflammatory response in a rabbit model of major trauma. J Trace Elem Med Biol 24(1):36-41. https://doi.org/10.1016/j.jtemb.2009.08.002

Yücel I, Arpaci F, Özet A et al (1994) Serum copper and zinc levels and copper/zinc ratio in patients with breast cancer. Biol Trace Elem Res 40(1):31-38. https://doi.org/10.1007/BF02916818

Vural H, Uzun K, Uz E, Koçyigit A, Cigli A, Akyol Ö (2000) Concentrations of copper, zinc and various elements in serum of patients with bronchial asthma. J Trace Elem Med Biol 14(2):88-91. https://doi.org/10.1016/S0946-672X(00)80036-X